Life History of the Endangered Fine-Rayed Pigtoe *Fusconaia cuneolus* (Bivalvia: Unionidae) in the Clinch River, Virginia

Sue A. Bruenderman[1] and Richard J. Neves

U. S. Fish and Wildlife Service, Virginia Cooperative Fish and Wildlife Research Unit[2], Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061-0321 U.S.A.

Abstract. The reproductive cycle, fish hosts, and selected population statistics of the endangered fine-rayed pigtoe *Fusconaia cuneolus* (Lea, 1840) were investigated during 1986-1987 in the Clinch River, Virginia. Examination of gravid females and drift samples indicated that the summer brooder is gravid from mid-May to late July and releases most glochidia in mid-June. Diel samples of stream drift contained a peak of glochidia in early morning. Of 1619 collected and examined fishes of 39 species for glochidial attachment, infestation by amblemine glochidia was highest on cyprinids (27 - 46%). Six species were identified as likely hosts for glochidia of the fine-rayed pigtoe. Induced infestations of fishes with glochidia in the laboratory confirmed eight host species: fathead minnow *(Pimephales promelas)*; river chub *(Nocomis micropogon)*; stoneroller *(Campostoma anomalum)*; telescope shiner *(Notropis telescopus)*; Tennessee shiner *(N. leuciodus)*; white shiner *(Luxilus albeolus)*; whitetail shiner *(Cyprinella galactura)*; mottled sculpin *(Cottus bairdi)*.

Age class and growth rate characteristics, determined by sectioning of valves collected in muskrat middens, were similar between two demes in the river. The fine-rayed pigtoe reaches a maximum length of about 90 mm and age of 35 yr. Annual growth averaged 5 mm/yr through age 10 and decreased to about 2 mm/yr thereafter until senescence. Specimens less than age 10 were uncommon, and no individuals under age 6 were collected. Cohort structure of live specimens and collections of valves indicated that the fine-rayed pigtoe population is declining in the Clinch River, Virginia.

The upper Tennessee River watershed in southwestern Virginia harbors a diverse assemblage of freshwater mussels (Unionidae) that seems to be in significant decline. Of the roughly 45 reported species from this region, 13 are listed as endangered by the federal government and another 20 are proposed for protection by Virginia (Neves, 1991). Declines in abundance and diversity of mussels are due largely to anthropogenic activities (Bates and Dennis, 1978; Ahlstedt, 1984). However, a consortium of state and federal agencies now are involved actively in the protection and recovery of biodiversity in these rivers.

The fine-rayed pigtoe pearly mussel *Fusconaia cuneolus* (Lea, 1840) was listed as endangered on 14 June 1976 (Federal Register 41:24062-24067). Historically this species was widespread in tributaries of the Tennessee River in Virginia, Tennessee, and Alabama, but now is restricted to reaches of seven rivers in the upper and lower Tennessee River system. The largest population occurs upstream of Norris Reservoir in the Clinch River, Virginia and Tennessee (Fig. 1). Ortmann (1921) noted that this species was a short-term brooder (tachytictic), breeding from May to July, and that all four gills served as marsupia for its glochidia, which are subelliptical in shape and of similar length and height (0.16 mm). Other aspects of its biology and life history were unknown. The objectives of this study, therefore, were to describe the chronology of the reproductive cycle, identify fish hosts, and determine the demographics of two demes of the fine-rayed pigtoe in the Clinch River, Virginia.

STUDY SITE

The principal study site was at Slant, Clinch River Mile (CRM) 223.3 in Scott County, Virginia (Fig. 2). From an

Fig. 1. Specimen of *Fusconaia cuneolus* from the Clinch River, Virginia.

[1]Present address: Virginia Department of Game and Inland Fisheries, 2206 South Main Street, Blacksburg, Virginia 24060

[2]The Unit is jointly supported by the United States Fish and Wildlife Service, the Virginia Department of Game and Inland Fisheries, the Wildlife Management Institute, and Virginia Polytechnic Institute and State University

American Malacological Bulletin, Vol. 10(1) (1993):83-91

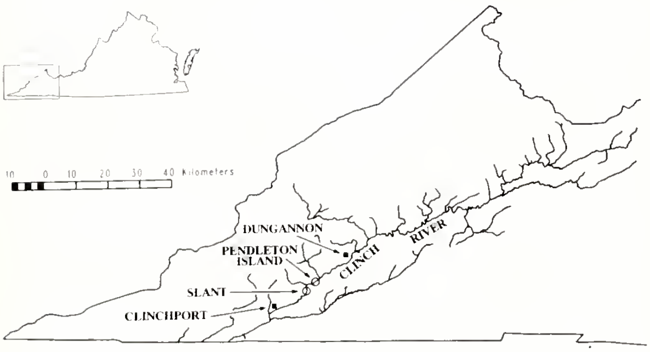

Fig. 2. Location of study sites on the Clinch River, Virginia.

assessment of muskrat predation on endangered mussels in the Clinch River, Neves and Odom (1989) recorded 32 mussel species and a large population of the fine-rayed pigtoe at this site. The river reach with *Fusconaia cuneolus* was approximately 325 m long and 50 m wide, with substrata of coarse gravel, cobbles, and bedrock. One additional site at Pendleton Island (CRM 226.3), a preserve of The Nature Conservancy, provided gravid females for glochidia and fresh-dead valves for demographic analysis.

MATERIALS AND METHODS

FIELD STUDIES

During the summers of 1986 and 1987, we monitored the incidence of gravid females at the study sites. Specimens of *Fusconaia cuneolus* were opened carefully with modified o-ring expanders to examine gills. Because this species is not sexually dimorphic in shell characters, gender could be determined only during the period of gravidity. Gravid females were identified by intensely pink and enlarged marsupial gills (Ortmann, 1921). Observations of weekly changes in coloration of female gills (marsupia) in 1986 prompted a more detailed examination in 1987. At Slant, examined females were placed under hardware mesh cloth in 1986 to prevent depredation by muskrats. In 1987, these stockpiled females were marked with 5 mm x 3 mm numbered disc tags (Floy Tag Co., Seattle, Washington[3]) to allow weekly monitoring of individuals. We recorded date, color of gills, and percent gravid females. Mid-day water temperatures were recorded at Slant with a max-min thermometer. Fecundity was computed from the number of conglutinates in a sacrified female (70 mm length) and the mean number of embryos per conglutinate.

Because the glochidia of *Fusconaia cuneolus* were similar in appearance to three other species of the subfamily Ambleminae at the study site, we collected and statistically compared glochidia from each of five specimens of these other amblemine mussel species: long-solid *F. subrotunda* (Lea, 1831); shiny pigtoe *F. cor* (Conrad, 1834); Tennessee clubshell *Pleurobema oviforme* (Conrad, 1834). Glochidia of mussel species in the subfamily Ambleminae are distinguishable by their characteristic size and shape (Neves and Widlak, 1988). Sample sizes were 50 glochidia for the *Fusconaia* spp. and 42 glochidia for *P. oviforme*. Expelled glochidia from gravid specimens were measured in length, width, and hinge lengths (Table 1). A Kruskal-Wallis test was used to determine whether valve dimensions differed among species. An LSD procedure with ranks identified those dimensions that were significantly different.

To determine the period of release of glochidia, we sampled stream drift weekly with three square-framed drift nets (0.045 m^2) of 130 um mesh nylon netting with removable cod-ends. Drift nets were staked into the river bottom downstream of a mussel bed on a line transect for roughly 1 hr between 1000 and 1500 hr on each sampling date. Drift samples were collected from 16 June to 13 Aug 1986, and 29 May to 30 July 1987. Samples were preserved in 10% formalin buffered with sodium borate to prevent dissolution of glochidial valves. In the laboratory, rose bengal was added to samples to facilitate recognition of glochidia. A 0.5 mm mesh sieve was used to remove large particulate matter, and finer debris and glochidia were collected in a 130 um mesh sieve. The latter was examined in a gridded petri dish with a dissecting microscope (25-40X) and glochidia were removed and counted. Densities of glochidia were computed from measurements of water depth and water velocity at the net opening and from counts of glochidia per sample.

The diel periodicity of glochidial release of *Fusconaia cuneolus* was determined at Slant on 16-17 June 1987, the recorded time of peak release in 1986. Every 4 hr during a 24 hr period, we positioned one drift net in the stream where the greatest densities of glochidia were collected in 1986. Numbers of glochidia were tabulated for each of the six time periods to compare densities in a 24 hr daily cycle.

The fish fauna at Slant was sampled for roughly 2 hr weekly in a 100 m section of the river with a backpack electroshocker and dip nets. Stunned fishes were collected, sorted and preserved in 10% buffered formalin. In the laboratory, opercular flaps were removed to examine gills with a dissecting microscope (20-40X). No amblemine glochidia were observed externally on the fishes. We tabulated frequency of occurrence and number of glochidia per fish. Fish species with encysted amblemine glochidia were considered possible hosts for the fine-rayed pigtoe.

LABORATORY INFESTATIONS

We confirmed fish hosts of the fine-rayed pigtoe in the laboratory by induced infestations of putative fish hosts with glochidia. Dechlorinated tap water, oxygenated by Venturi-type diffuser aerators, was recirculated through a central

[3]Reference to trade names does not imply government endorsement of commercial products.

Table. 1. Mean dimensions (mm) ± SD and morphometric comparisons of glochidia of selected amblemine species from the Clinch River, Virginia.

Glochidial dimension	*Pleurobema oviforme*	*Fusconaia cuneolus*	*F. cor*	*F. subrotunda*
Length	0.185 ± 0.007	0.181 ± 0.008	0.181 ± 0.006	0.181 ± 0.009
Width	0.193 ± 0.004	0.193 ± 0.005	0.146 ± 0.006	0.150 ± 0.028
Hinge length	0.150 ± 0.005	0.165 ± .009	0.122 ± 0.005	0.118 ± 0.005

Underscore indicates no difference between species ($p \geq 0.05$).

gravel biofilter and 96 L rectangular tanks. Fishes for induced infestations were collected from river reaches in Virginia, where mussels were absent or rare, to eliminate the possibility of acquired immunity from prior exposure to glochidia (Neves *et al.*, 1985). Target fish species were electroshocked, scooped with a bucket, and transported in coolers containing aerated river water and 2% salt solution. Ice packs were added when necessary to maintain water temperatures (20°-26°C) comparable to those in laboratory holding tanks.

Prior to infestations, collected fishes were acclimated for approximately five days. A 4% benzocaine solution was used to anesthetize fishes and facilitate the identification of cyprinids. Fishes were sorted by species into separate tanks, and the tops of standpipes were covered with 5 mm mesh screen to prevent escape.

Mature glochidia were obtained from gravid females collected at Pendleton Island and Slant, Virginia. Mussels were transported to the laboratory and induced to abort conglutinates by placing them in individual 400 ml beakers without substratum. Beakers were set in a Living Stream (Frigid Units, Inc., Toledo, OH) at a mean temperature of 22°C until glochidia were expelled. If females failed to release glochidia after five days, the beakers were removed from the stream and held at room temperature (23°C). The increased temperature and static water were usually effective in promoting expulsion of glochidia. Conglutinates were retrieved with a large-bore pipette, and glochidia were extricated from the conglutinate by repeated agitation in a finger bowl. Infestations were performed with three containers to hold, anesthetize, and recover infested fishes. Procedures for infestation, examination of fishes, and recovery of juveniles were performed according to Zale and Neves (1982). Juveniles were identified by their yellowish color, thickened ventral margins, and movement of the protruding foot. A sample of juveniles was relaxed in propylene phenoxitol, preserved in 10% buffered formalin, and later photographed. A fish species was considered a host of the fine-rayed pigtoe if encystment and metamorphosis to the juvenile stage occurred. Most laboratory tests that confirmed host fish species in 1986 were repeated in 1987.

AGE AND GROWTH

Valves of the fine-rayed pigtoe, collected at Pendleton Island and Slant, were measured and aged by thin-sectioning with a Buehler Isomet low-speed diamond-tipped saw (Buehler Ltd., Evanston, Illinois). Five to ten valves per length group (10 mm intervals) from each site were thin-sectioned (Neves and Moyer, 1988). Age of each specimen was determined by counting internal growth lines on thin-sections using a compound microscope (50X). The point where the annulus reached the valve margin was marked with a black felt-tipped pen. The marked thin-sections then were held facing the cross-section from which the cut was produced. Internal growth lines were compared with external growth lines, and false annuli were identified (Neves and Moyer, 1988). Because specimens younger than age nine were rare, we obtained back-measured lengths of younger age groups. On five specimens from each site, external annuli on their cross-sections were marked. By overlaying this valve section onto the matching uncut valve, we measured lengths of the growth lines at previous ages. For these back-measured lengths, we used valves with minimal erosion and easily distinguished annuli.

Total lengths by age were obtained from thin sections and fitted to a modified version of the von Bertalanffy growth equation (Gallucci and Quinn, 1979):

$$L_t = (w/k)\ (1-e^{-k\,(t-t_o)})$$

where t is a given age in years, t_o is the theoretical time when length is zero, k is a growth constant,and w describes growth rate near t_o. The parameter w is the product of K and $L\infty$ and replaces $L\infty$ in the original von Bertalanffy equation. Gallucci and Quinn (1979) concluded that this new parameter (w), because of its statistical robustness, allows comparison of growth rates among populations. Non-linear procedures were applied to derive estimates of w, k, and t_o. Based on these values, estimates of L_t were computed (SAS Institute, 1982). Confidence intervals of parameter estimates were compared for overlap to determine differences in growth of *Fusconaia cuneolus* between sites. The equation of predicted lengths at each age, derived from thin-sectioned valves, was used to predict ages from total lengths of unaged specimens.

We obtained lengths from valves of *Fusconaia cuneolus* collected in muskrat middens at Slant from 1980 to 1986. Ages of 353 mussels in this collection were computed by age-length relationships, and the age-class distribution of the deme at this site was determined. Lengths of 43 live specimens, randomly collected at Slant in 1987, also were converted to ages to determine age class structure. The two age distributions were compared with the G-test (R X C test of independence) to determine whether collection method (human vs muskrat) influenced interpretation of sampling results.

Nomenclature for mussels is according to Turgeon *et al.* (1988). Binomials for fishes follow Robins *et al.* (1991).

RESULTS

REPRODUCTIVE CYCLE

Gravid females of the fine-rayed pigtoe were collected first on 16 June 1986. Four of eight specimens were gravid. Between 16 July and 6 Aug 1986, percent gravidity was high (50-100%), but sample size on each date was small (N = 4). However, a random sample of 33 fine-rayed pigtoes collected at Pendleton Island on 25 July also indicated that many females (61%) were gravid during this time. One female was gravid at Slant on 6 Aug. No gravid specimens were collected thereafter.

In 1987, two gravid females were collected on 10 June (Fig. 3). Most of the stockpiled females at Slant were collected again on 18 June 1987. Gravid specimens were collected as late as 6 Aug 1987 at Pendleton Island, which corroborated our observations in 1986. Females began incubating eggs in

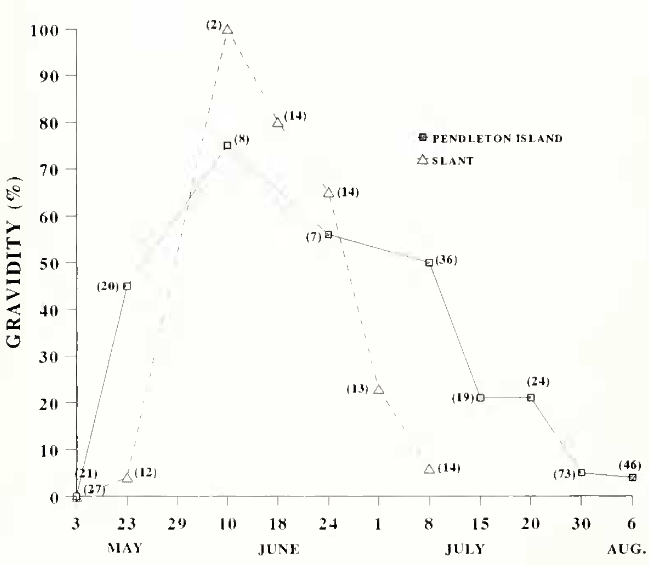

Fig. 3. Frequency of gravid females in the Clinch River, Virginia, during summers, 1987 and 1988 combined (sample sizes in parentheses).

Table 2. Water temperatures and densities of glochidia of the fine-rayed pigtoe in weekly drift samples from the Clinch River at Slant, Virginia, 1986 and 1987.

Sample date	Temperature range (°C)	Weekly median temperatures (°C)	Glochidial densities (no/100m³)
1986			
6/16	—	25.4	43.0
6/25	21.4-27.4	24.4	1.9
7/01	22.4-27.9	25.1	6.6
7/09	23.4-27.9	25.6	27.0
7/16	24.4-28.4	26.4	39.7
7/25	25.4-29.9	27.6	8.9
7/30	24.4-29.4	26.9	9.4
8/05	21.4-28.4	24.9	2.9
8/13	22.9-27.4	25.1	3.9
1987			
5/29	—	25.4	1.7
6/10	—	23.0	54.7
6/18	22.9-24.9	23.0	126.6
6/24	23.4-25.1	24.0	38.3
7/01	23.0-25.6	23.5	20.8
7/08	22.0-24.3	23.0	60.3
7/16	21.6-24.2	23.5	3.9
7/20	21.9-24.0	23.3	7.9
7/30	25.0-27.4	26.0	0.8
8/05	26.2-28.0	26.8	—

early to mid-May, as judged by the additional collection and examination of females in 1988. Females were not gravid at Pendleton Island (N = 21) or Slant (N = 27) on 3 May, but by 23 May, one of 12 gravid females examined at Slant and nine of 20 at Pendleton Island were gravid. Mid-day water temperatures at Slant increased from 16° to 21°C during this time period.

The marsupia of all gravid females collected on 10 June and 4 July contained peach-colored conglutinates. By frequent examination of females at Slant and those brought to the laboratory, we noted a change in marsupial color with maturity - from pink to orange to light peach. In the laboratory, females with light peach marsupia consistently released mature glochidia, held together in a loose gelatinous matrix. Females released mature glochidia within seven to 14 days after the pink color was observed, at water temperatures between 20° and 24°C. Development from embryo to glochidium required roughly 2 wk within this temperature range.

Mature glochidia were light orange to peach in color and free of vitelline membranes. Unfertilized eggs, embryos, and immature glochidia were distinctively pink, but unfertilized eggs lacked vitelline membranes. When aborted, immature glochidia were enclosed in tightly organized, pink conglutinates. Marsupia of females, immediately following release of glochidia, exhibited a bubbly appearance and were less turgid than the gills of gravid females.

Table 3. Incidence of glochidial infestations on cyprinid species from the Clinch River, Virginia, 17 June to 30 July, 1987.

Species	No. examined	No. infested		% infested	
		Ambleminae	Other	Ambleminae	Other
Notropis amblops (Rafinesque, 1820)	265	1	26	0.4	9.8
N. volucellus (Cope, 1865)	199	54	44	27.1	22.1
Campostoma anomalum (Rafinesque, 1820)	88	7	5	8.0	5.7
Luxilus chrysocephalus Rafinesque, 1820	59	4	8	6.8	13.6
N. sp.	48	22	12	45.8	25.0
N. ariommus (Cope, 1868)	46	1	2	2.2	4.3
Nocomis micropogon (Cope, 1865)	45	14	3	31.1	6.7
Erimystax dissimilis (Kirtland, 1840)	41	0	0	0.0	0.0
Cyprinella galactura (Cope, 1868)	33	13	8	39.4	24.2
Notropis telescopus (Cope, 1868)	31	2	1	6.7	3.2
L. coccogenis (Cope, 1868)	30	2	1	6.7	3.3
N. leuciodus (Cope, 1868)	20	6	2	30.0	10.0
E. insignis (Hubbs and Crowe, 1956)	18	0	0	0.0	0.0
C. spiloptera (Cope, 1868)	13	4	2	30.8	15.4
Pimephales notatus (Rafinesque, 1820)	8	1	0	12.5	0.0
Phenacobius uranops Cope, 1867	8	0	0	0.0	0.0
N. photogenis (Cope, 1865)	7	0	0	0.0	0.0
C. whipplei Girard, 1856	4	0	2	0.0	5.0
N. rubellus (Agassiz, 1850)	2	0	0	0.0	0.0
Total	965	131	116		

For host fish experiments, only females with peach-colored marsupia were transported to the laboratory. The 41 gravid females held in the laboratory during 1987 released glochidia from 14 June to 11 Aug at water temperatures between 20° and 24°C. Thirty-three females released conglutinates with nearly 99% mature, viable glochidia. Eight females released conglutinates with 3 to 5% unfertilized eggs. Immature glochidia were released by only one female on 19 July.

Conglutinates were subcylindrical in shape and approximately 6 mm long and 1.5 mm wide with two layers of tightly aggregated glochidia about 0.8 mm deep. One conglutinate from each of five females contained a mean of 236 $\pm$ 38.1 embryos or glochidia. Fecundity was about 113,000 embryos for the sacrificed female.

Hinge lengths differed among each of the four amblemine species (Table 1; $p = 0.0001$, $n = 42\text{-}50$ glochidia per species). Glochidia of *Fusconaia cuneolus* were greater in width than those of *F. cor* and *F. subrotunda*, but were not different from those of *Pleurobema oviforme.* Total lengths of glochidia of all species did not differ. The glochidia of the fine-rayed pigtoe most closely resembled those of *P. oviforme*, but were visually distinguished by a longer hinge length and more rotund appearance.

We observed two peaks in the presence of glochidia in 1986 and 1987 (Table 2). In 1986, peak densities of glochidia occurred in mid-June (43.0/100 m^3) and mid-July (39.7/100 m^3). A similar trend in densities was observed in 1987, although the second peak occurred one week earlier than in 1986. Multiple linear regression analyses indicated that densities of glochidia were not correlated with date of sample, weekly median temperature prior to sample date, or water velocity at the drift net.

From 16 to 17 July 1987, we collected glochidia of *Fusconaia cuneolus* in stream drift throughout the 24 hr period, at water temperatures ranging from 21.2° to 23.0°C. Densities of glochidia were lowest at 2300 and 0300 hr (1/100 m^3) and peaked at 0700 hr (118/100 m^3). Water velocity at the drift net was constant at 0.4 m/sec for the period.

FISH HOSTS

From 17 June to 30 July 1987, we collected and examined 1,150 fishes representing 39 species at Slant for attached glochidia. Thirteen fish species, all cyprinids, were infested with glochidia of the Ambleminae (Table 3). The highest prevalence of infestation on cyprinids occurred on 17 June (17%) and 8 July (25%) 1987 (Table 4), dates corresponding to the highest densities of glochidia of *Fusconaia cuneolus* in stream drift. Minnow species with the highest incidence of infestation were the sawfin shiner *Notropis* sp. (45.8%), whitetail shiner *Cyprinella galactura* (Cope, 1868) (39.4%), river chub *Nocomis micropogon* (Cope, 1865) (31.1%), spotfin shiner *C. spiloptera* (Cope, 1868) (30.8%), Tennessee shiner *Notropis leuciodus* (Cope, 1868) (30%) and mimic shiner *N. volucellus* (Cope, 1865) (27.1%). These fish species were identified as the most probable hosts for the fine-rayed pigtoe.

On various sample dates in 1987, glochidia identified as *Fusconaia cuneolus* were found encysted in the gills of four species of cyprinids; river chub, whitetail shiner, stoneroller,

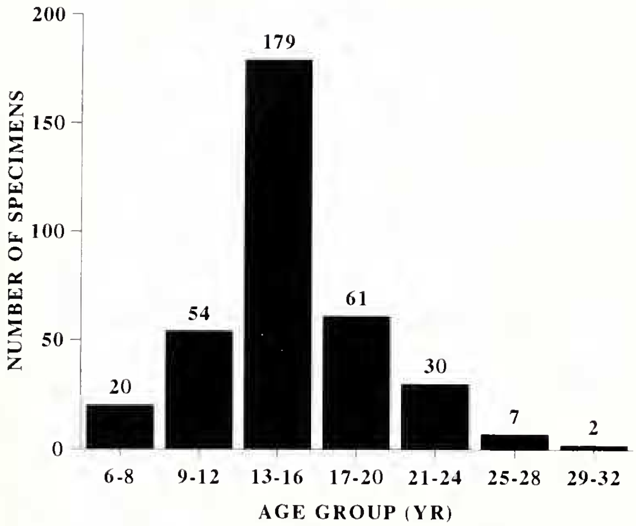

Fig. 4. Age class structure of fine-rayed pigtoes collected in muskrat middens from the Clinch River at Slant, Virginia, 1984-85.

and spotfin shiner. Newly metamorphosed juveniles of the fine-rayed pigtoe were observed on the gills of river chubs on all weekly sample dates between 8 and 30 July 1987. Gills of spotfin shiners also harbored newly metamorphosed juveniles of *F. cuneolus* on 21 and 30 July. These young juveniles, easily removed from cysts with a dissecting needle, exhibited the typical yellowish color and thickened ventral margins. The degree of infestation of the fine-rayed pigtoe on individual fish ranged from one to 26 glochidia. Numbers of glochidia per fish were highest for the river chub (1-26 glochidia/fish); however, most infested fishes carried one to five glochidia per individual.

In laboratory exposures, glochidia of *Fusconaia cuneolus* were sloughed from the gills of eight of the 16 fish species tested (Table 5). Seven infested non-cyprinid species were not considered hosts because they sloughed glochidia usually within one to five days. However, the fantail darter *Etheostoma flabellare* Rafinesque, 1819 retained glochidia for nine days, and the goldfish *Carassius auratus* (Linnaeus, 1758), for six days. The goldfish was the only examined minnow that did not serve as a host.

Seven species of cyprinids and the mottled sculpin *Cottus bairdi* Girard, 1850 were confirmed as fish hosts (Table 6). The periods of metamorphosis ranged from 10 to 19 days at mean water temperatures between 22.5^0 and 25.0^{0}C. At mean water temperatures of about 23^{0}C, most juveniles were collected 15 to 16 days post-infestation. Peak numbers of juveniles were collected from river chubs on day 11 at 24^{0}C and from mottled sculpins on day 13 at 25^{0}C.

AGE AND GROWTH

The pattern of growth in shell length of the fine-rayed pigtoe was asymptotic. Mean annual growth in length averaged 5.0 mm/yr before age six, 3.7 mm/yr between ages six and 16, and 2.1 mm/yr between ages 16 to 21 (Table 7). Thereafter, growth slowed to roughly 1 mm/yr. Growth equations describing increases in length were as follows:

$$L_t = (10.4/0.13)\ (1 - e^{-0.13\ (t\ -\ 0.05)}) \text{ at Pendleton Island;}$$
$$L_t = (\ 7.3/0.08)\ (1 - e^{-0.08\ (t\ -\ 0.031)}) \text{ at Slant.}$$

Except for more rapid growth of juveniles up to age five at Pendleton Island, all subsequent growth increments were similar between the two sites.

The ages of live specimens of fine-rayed pigtoes at both sites ranged from six to 32 years; individuals 17 to 20 years old were the dominant age groups (Table 8). The most common specimens found in muskrat middens (Fig. 4) were 13 to 16 years old. Younger specimens (< age 10) were more common in muskrat middens than in the sample of live specimens collected by snorkeling and handpicking. Similarly,

Table 4. Dates of infestation of amblemine glochidia on cyprinids from the Clinch River at Slant, Virginia, summer 1987.

Sample date	No. examined	No. infested	% infested
17 June	102	17	17
25 June	71	7	10
8 July	118	29	25
16 July	184	27	15
21 July	305	34	11
30 July	179	18	10
Total	959	122	

Table 5. Fish species that did not serve as hosts for the glochidia of the fine-rayed pigtoe in laboratory experiments.

Infested species	No. infested	Temperature range (^{0}C)	Last day of observed attachment
Centrarchidae			
Lepomis macrochirus Rafinesque, 1819	4	21.0-24.0	5
L. auratus (Linnaeus, 1758)	1	20.0-25.0	5
Ambloplites rupestris (Rafinesque, 1817)	5	24.0-26.0	2
Cyprinidae			
Carassius auratus (Linnaeus, 1758)	10	24.0-25.0	6
Ictaluridae			
Ictalurus punctatus Rafinesque, 1818)	6	24.0	1
Noturus insignis (Richardson, 1836)	4	24.0	1
Percidae			
Etheostoma flabellare Rafinesque, 1819	7	24.0-26.0	9
E. rufilineatum (Cope, 1870)	10	24.0-26.0	4

a greater percentage of old individuals were included in the collection of live mussels (X^2 = 76.6, 2 df, p = 0.0001, n = 580). Young specimens were rarely found at either site, but several specimens (age six) were collected in muskrat middens at Slant.

DISCUSSION

Our conclusion that the fine-rayed pigtoe is a short-term brooder and gravid from mid-May to early August in the Clinch River corroborates Ortmann's (1921) records of gravidity (16 May - 13 July) for this species in the upper Tennessee River drainage and is in general agreement with the chronology of reproduction by other short-term brooders (Yokley, 1972; Yeager and Neves, 1986). The fine-rayed pigtoe appears to spawn and incubate eggs when water temperatures reach 16°C. Seasonal change in temperature is often noted as an important environmental cue for spawning of mussels (Coker *et al.*, 1921; Matteson, 1948; Yokley, 1972; Smith, 1976). The above-normal air temperatures and below-normal precipitation during summer 1986 (Virginia Agricultural Statistics Service, 1987) could account for differences we observed in the reproductive cycle between 1986 and 1987.

We were able to follow the development of embryos by monitoring color changes of the marsupia (conglutinates within). Yeager and Neves (1986) noted color changes of the marsupia of the rough rabbitsfoot *Quadrula cylindrica strigillata* (Wright, 1898), and this trait seems useful for categorizing the maturity of glochidia. As judged by observed color changes, the development of fertilized eggs to glochidia requires approximately 2 wk for fine-rayed pigtoes. Because early embryos occur in female marsupia shortly after spawning (Coker *et al.*, 1921; Zale and Neves, 1982), we postulate that *Fusconaia cuneolus* spawns in early May. Histological studies of other amblemine species support this conclusion (Matteson, 1948; Yokley, 1972; Yeager and Neves, 1986).

Glochidia of the fine-rayed pigtoe exhibited considerable host specificity, primarily to fishes of the Cyprinidae. The relationship between short-term brooders and cyprinids appears to apply throughout the upper Tennessee River drainage (Yeager and Neves, 1986; Neves and Widlak, 1988). As judged by the prevalence of natural infestations on the river chub, whitetail shiner, and Tennessee shiner, we believe that these fish species are primary hosts for the fine-rayed pigtoe in the Clinch River. Further, these fish species are widespread and common in the Clinch River and other tributaries to the upper and lower Tennessee River. The use of multiple hosts by a freshwater mussel in a dynamic, lotic environment has obvious survival value (Trdan and Hoeh, 1982; Kat, 1984). Because the genera *Notropis* and *Cyprinella* tend to dominate the cyprinid assemblages in most southeastern rivers, confirmed hosts and congeneric species in rivers with the fine-rayed pigtoe probably serve as hosts. Subsequent research on host fishes with other endangered populations of *Fusconaia cuneolus* should focus on the cyprinid assemblage in their respective rivers.

We recorded only one to five glochidia on most naturally infested fishes, and this low level of infestation is seemingly typical of riverine mussel species (Neves and Widlak, 1988). Early studies concluded that fish species with few encysted glochidia were probably incidental hosts (Surber, 1912; Howard and Anson, 1922; Tedla and Fernando, 1969), but low degrees of infestation seem to be the rule rather than the exception. River chubs and *Notropis* spp. commonly feed on drifting insects and could become infested by feeding on released conglutinates in the water column and, therefore, experience a higher degree of infestation (Neves and Widlak, 1988). Conversely, the stoneroller is a grazing minnow that feeds principally by scraping aufwuchs from rocks and other substrata. Although both feeding groups can become infested (Table 3), our data suggest that cyprinids feeding visually in the water column are the primary fish hosts for the fine-rayed

Table 6. Fish species that served as hosts for the glochidia of the fine-rayed pigtoe in laboratory experiments.

Infested species	No. infested	Period of metarmorphosis	Peak day of excystment	Temperature range (°C)	No. juveniles recovered
Cyprinidae					
Pimephales notatus	10	12-16	15	21.0-26.0	76
Nocomis micropogon	2[1]	11-19	11	22.0-25.5	50
Campostoma anomalum	3	12-16	15	21.0-26.0	537
Notropis telecopus	8	10-16	15	22.0-23.5	101
N. leuciodus	9[2]	13-14	—	22.0-23.0	2
Luxilus albeolus	6	10-18	16	21.0-26.0	424
Cyprinella galactura	10	12-16	16	22.0-23.5	760
Cottidae					
Cottus bairdi	7	12-14	13	24.0-26.0	84

[1]Experiment performed in 1986

[2]All died from disease prior to completion of experiment

Table 7. Sample size and observed and predicted lengths of the fine-rayed pigtoe in the Clinch River at Slant, Virginia.

Age	N	Observed length mean ± 1 s.d.	Predicted length
1	10	8.6 ± 1.7	5.0
2	10	12.9 ± 1.9	11.6
3	9	18.7 ± 4.2	24.8
4	10	24.0 ± 4.5	23.3
5	10	28.3 ± 3.8	28.5
6	11	32.7 ± 4.4	33.2
7	11	37.6 ± 5.9	37.7
8	10	37.8 ± 2.6	41.8
9	2	43.9 ± 1.9	45.5
10	8	46.1 ± 7.6	49.0
11	2	55.5 ± 8.7	52.5
12	8	56.1 ± 5.0	55.2
13	6	57.4 ± 7.8	58.0
15	5	65.3 ± 5.0	62.8
16	3	68.6 ± 6.8	65.0
17	1	71.7 —	67.0
18	1	74.8 —	68.8
20	2	73.5 ± 3.8	72.1
23	1	65.9 —	76.1
25	1	77.0 —	78.2
27	1	83.6 —	80.1
30	1	80.2 —	82.4

pigtoe.

The mottled sculpin was the only non-cyprinid that served as host for the fine-rayed pigtoe. This species was not collected at Slant. Although sculpins are ubiquitous in the Tennessee River system, the mottled sculpin is rare whereas the banded sculpin is common throughout the upper Clinch River. Neves *et al.* (1985) determined that congeneric, allopatric species can serve as fish hosts; therefore, the suitability of *Cottus* spp. as hosts is likely. Weaver (1981) documented that the banded sculpin served as host for the Tennessee clubshell in laboratory trials, but she observed no natural infestations on it in Big Moccasin Creek, Virginia. Therefore, sculpins seem to be suitable but not primary hosts for short-term brooders in southwest Virginia.

Because the fine-rayed pigtoe is a slow-growing species with frequent erosion of umbos, examination of thin-sections under magnification was essential for accurate aging. On average, 1 to 1.5 hr was required to prepare each valve, and an additional 12 hr for drying the epoxy. Recognition of false annuli was apparent on thin sections (Neves and Moyer, 1988). The growth pattern for the fine-rayed pigtoe at Pendleton Island was very similar to that reported by Moyer (1984) for a downstream deme of this species. Asymptotic lengths and estimates of K were comparable, but values of t_0 were different. This discrepancy is most likely explained by estimates of length at age one. Eroded umbos usually obliterate the first annulus, and estimates are often best guesses. Mean lengths of some cohorts were underestimated by the growth equation, but they concurred with empirical data when sample sizes were greater than five mussels. Because we were unable to obtain suitable numbers of both old and young mussels for aging, predicted lengths at these ages could be more accurate than observed values.

Theoretical maximum lengths ($L\infty$) and estimated values of K were different between demes at Slant and Pendleton Island, but these parameters are unsuitable for comparison among populations (Gallucci and Quinn, 1979; Haukioja and Hakala, 1979). However the parameter w, which is suitable for comparison (Gallucci and Quinn, 1979), was significantly different between sites. Fine-rayed pigtoes grew faster at Pendleton Island than at Slant. We attribute this difference to the shallower water depths and greater water velocities at Pendleton Island that probably increased oxygen levels and the availability of food particles per unit time for filter-feeders.

The age range (6 to 32) of fine-rayed pigtoes and the dominance of few, older cohorts in this reach of the Clinch River were unexpected. The population of *Fusconaia cuneolus* in the Clinch River is considered to be the largest and healthiest (U.S. Fish and Wildlife Service, 1984). Although freshwater mussel populations are dominated commonly by older cohorts, sampling techniques often contribute to that age (size) bias (Chamberlain, 1931; Coon *et al.*, 1977). Our sampling results suggested that because of the predation and apparent selectivity of fine-rayed pigtoes over other species by muskrats at Slant (Neves and Odom, 1989), determination of age-class structure from specimens in muskrat middens provided a reasonable assessment of population demographics. The lack of fine-rayed pigtoes < age 10 in a large sample (N = 264) from muskrat middens indicated that recruitment is poor. Similarly, the reduction in population size from muskrat predation has been large (Neves and Odom, 1989). As computed from the age-length relationship for the fine-rayed pigtoe, ages 11 to 14 (56-65 mm) were consumed by muskrats with greatest frequency. The removal of members of these age groups and the rare occurrence of mussels < age 10 has reduced the reproductive potential and likely will jeopardize the continued abundance of this species

Table 8. Comparison of age class structure of live specimens and fresh-dead valves (muskrat middens) of the fine-rayed pigtoe from the Clinch River, Virginia[1].

	Midden Shells		Live Specimens	
Age Class	No.	Percent	No.	Percent
< 10	79	22.4	8	3.5
11-20	263	74.5	176	77.5
21-30	11	3.1	43	19.0
Total	353	100	227	100

[1]RXC test of independence with the G-test with Williams correction (X^2 = 76.6, 2 df, p = 0.0001).

at study sites in the Clinch River. Recent sampling and monitoring of mussels at various sites on the Clinch River, Virginia suggest that the fine-rayed pigtoe and other mussel species are declining in abundance in most reaches of the Clinch River. A new cooperative effort by governmental agencies and conservation organizations is underway to identify and remedy the factors that are contributing to the loss of freshwater mussel populations in this river.

ACKNOWLEDGMENTS

We thank Kurt Jirka, Lisie Kitchel, and Bob Sheehan for assistance in the initial stages of the project. Lisa Wolcott, Becky Wajda, Joe Stoeckel, and Tim Simonson were especially helpful in field and laboratory studies. We also acknowledge Paul Angermeier and Don Orth for their contributions to the analysis of field data. Finally, we thank the U.S. Forest Service, Monongahela National Forest, for the flexibility in work hours given to the senior author during manuscript preparation.

LITERATURE CITED

Ahlstedt, S. A. 1984. Twentieth century changes in the freshwater mussel fauna of the Clinch River. Master's Thesis, University of Tennessee, Knoxville, Tennessee. 102 pp.

Bates, J. M. and S. D. Dennis. 1978. The mussel fauna of the Clinch River, Tennessee and Virginia. *Sterkiana* 69/70:3-23.

Chamberlain, T. K. 1931. Annual growth of freshwater mussels. *Bulletin of the United States Bureau of Fisheries* 46:713-739.

Coker, R. E., A. F. Shira, W. H. Clark and A. D. Howard. 1921. Natural history and propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries* 37:771-181.

Coon, T. J., J. W. Ecklad and P. M. Trystad. 1977. Relative abundance and growth of mussels (Mollusca: Eumellibranchia) in pools 8, 9, and 10 of the Mississippi River. *Freshwater Biology* 7:279-285.

Gallucci, V. F. and F. J. Quinn, II. 1979. Reparameterizing, fitting and testing a simple growth model. *Transactions of the American Fisheries Society* 108:14-25.

Haukioja, E. and T. Hakala. 1979. Asymptotic equations in growth studies - an analysis based on *Anodonta piscinalis* (Mollusca, Unionidae). *Annales Zoologica Fennici* 16:115-122.

Howard, A. D. and B. J. Anson. 1922. Phases in the parasitism of the Unionidae. *Journal of Parasitology* 9:68-85.

Kat, P. W. 1984. Parasitism and the Unionacea (Bivalvia). *Biological Reviews* 59:189-207.

Matteson, M. P. 1948. Life history of *Elliptio complanatus* (Dillwyn, 1817). *American Midland Naturalist* 40:690-723.

Moyer, S. N. 1984. Age and growth characteristics of selected freshwater mussels from southwestern Virginia, with an evaluation of mussel aging techniques. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 176 pp.

Neves, R. J. 1991. Mollusks: *In: Virginia's Endangered Species: Proceedings of a Symposium.* K. Terwilliger (coordinator). pp. 251-320. McDonald and Woodward Publishing Company, Blacksburg, Virginia.

Neves, R. J., L. R. Weaver and A. V. Zale. 1985. An evaluation of host fish suitability for glochidia of *Villosa vanuxemi* and *Villosa nebulosa*. *American Midland Naturalist* 113:13-19.

Neves, R. J. and S. N. Moyer. 1988. Evaluation of techniques for age determination of freshwater mussels (Unionidae). *American Malacological Bulletin* 6:179-188.

Neves, R. J. and J. C. Widlak. 1988. Occurrence of glochidia in stream drift and on fishes of the upper North Fork Holston River, Virginia. *American Midland Naturalist* 119:111-120.

Neves, R. J. and M. C. Odom. 1989. Muskrat predation on endangered freshwater mussels in Virginia. *Journal of Wildlife Management* 53:934-941.

Ortmann, A. E. 1921. The anatomy of certain mussels from the upper Tennessee. *Nautilus* 34:81-91.

Robins, C. R., R. M. Bailey, C. E. Bond, J. R. Brooker, E. A. Lachner, R. N. Lea and W. B. Scott. 1991. Common and scientific names of fishes from the United States and Canada. *American Fisheries Society Special Publication* 20. 183 pp.

SAS Institute. 1982. *SAS User's Guide: Statistics.* Statistical Analysis System Institute, Cary, North Carolina. 584 pp.

Smith, D. G. 1976. Notes on the biology of *Margaritifera m. margaritifera* (Lin.) in northeastern North America. *Bulletin of the American Malacological Union* for 1976:49-53.

Surber, T. 1912. Identification of the glochidia of freshwater mussels. *Bulletin of the United States Bureau of Fisheries* 77:1-10.

Tedla, S. and C. H. Fernando. 1969. Observation on the glochidia of *Lampsilis radiata* (Gmelin) infesting yellow perch, *Perca falvescens* (Mitchell) in the Bay of Quinte, Lake Ontario. *Canadian Journal of Zoology* 47:705-712.

Trdan, R. J. and W. R. Hoeh. 1982. Eurytopic host use by two congeneric species of freshwater mussel (Pelecypoda: Unionidae: Anodonta). *American Midland Naturalist* 108:381-388.

Turgeon, D. D., A. E. Bogan, E. V. Coan, W. K. Emerson, W. G. Lyons, W. L. Pratt, C. F. E. Roper, A. Scheltema, F. G. Thompson and J. D. Williams. 1988. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. *American Fisheries Society Special Publication No. 16.* 277 pp.

United States Fish and Wildlife Service. 1984. *Fine-rayed Pigtoe Pearly Mussel Recovery Plan.* United States Fish and Wildlife Service, Atlanta, Georgia. 67 pp.

United States Federal Register. 1976. Endangered and threatened wildlife and plants. 41:24062-24067.

Virginia Agricultural Statistics Service. 1987. *Virginia Agricultural Statistics Bulletin No. 56.* Richmond, Virginia. 96 pp.

Weaver, L. R. 1981. Life history of *Pleurobema oviforme* (Mollusca: Unionidae) in Big Moccasin Creek, Virginia with emphasis on early life history, species association, and age and growth. Master's Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 90 pp.

Yeager, B. L. and R. J. Neves. 1986. Reproductive cycle and fish hosts of the rabbit's foot mussel *Quadrula cylindrica strigillata* (Mollusca: Unionidae) in the upper Tennessee River drainage. *American Midland Naturalist* 116:329-340.

Yokley, P. Jr. 1972. Life history of *Pleurobema cordatum* (Rafinesque, 1820) (Bivalvia: Unionacea). *Malacologia* 11:351-364.

Zale, A. V. and R. J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. *Canadian Journal of Zoology* 60:2535-2542.

Date of manuscript acceptance: 4 May 1992